

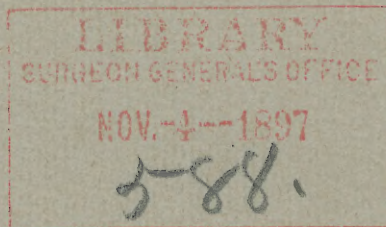
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FROM  
THE JOURNAL OF EXPERIMENTAL MEDICINE  
VOL. I, No. 4, 1896





# THE IMMUNIZING POWER OF NUCLEOHISTON AND OF HISTON.

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## HISTORICAL.

IN 1892 Lilienfeld\* showed that the nuclei of the lymphocytes of the thymus gland contained a characteristic body, which he first termed leuconuclein. A subsequent study revealed that this compound was not a true nuclein, but that it contained, in addition to nuclein, another body—histon. It was accordingly renamed as nucleohiston. The nuclei of cells, therefore, contain a very complex proteid compound—nucleohiston—which contains nuclein and histon in a saltlike combination.

Before Lilienfeld's work several investigators undoubtedly experimented with what was more or less pure nucleohiston. Thus, Wooldridge's tissue fibrinogen, which he obtained from the thymus and the testicle, is largely nucleohiston. Wooldridge prepared his tissue fibrinogen by precipitating with an acid the aqueous extract of the gland. The precipitate thus obtained was washed and finally dissolved in dilute sodium hydrate or carbonate. This method, it will be observed, is essentially the same as that employed subsequently by Lilienfeld. As is well known, with this tissue fibrinogen Wooldridge obtained some unique results.

In the first place, an injection of the alkaline solution of tissue fibrinogen intravenously in rabbits produced total thrombosis of the venous system. In dogs, however, this thrombosis was observed only

\* The references to literature cited in the text are at the end of this article.

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in special places, such as the track of the portal vein, and usually recovery took place. In the animals that recovered a much larger amount could be injected a second time without effect. This condition of non-coagulability as a result of the first injection was observed to persist for several days. When boiled, the fibrinogen solution lost the power of producing coagulation within the blood vessels, although it still coagulated pepton-plasma. These results have been confirmed and enlarged by Lilienfeld in his work with nucleohiston. The two components of this compound, however, were shown to possess opposite and antagonistic properties. Thus, nuclein promptly induced coagulation intravenously or in extravascular blood, such as pepton-plasma. Histon, on the other hand, injected intravenously, produced a non-coagulable condition of the blood.

With his fibrinogen solution Wooldridge further obtained some results on immunity from anthrax which at the time attracted no little attention. According to the degree of alkalinity, the fibrinogen solution on boiling either coagulated or became opalescent, or was scarcely altered. The last result was obtained with strongly alkaline solutions. In such the anthrax bacillus grew well, but, when filtered through paper and injected intravenously into rabbits, failed of effect. In the feebly alkaline solutions, which therefore were opalescent or partly coagulated, the anthrax germ grew poorly; but when such cultures were sterilized by heat and injected into the veins of rabbits, immunity resulted in eight out of nine animals. Subcutaneous injection of the sterilized fibrinogen culture fluid did not protect against subcutaneous anthrax, whereas intravenous application did. In another series of experiments he obtained immunity in a limited number of animals by the injection of boiled fibrinogen solution wholly devoid of bacterial products. This was one of the first instances where immunity was secured with a non-bacterial substance. The fibrinogen solution was boiled slightly, so that when squeezed through muslin an opalescent fluid with suspended particles resulted. With such liquids Wooldridge immunized two rabbits from anthrax.

These experiments of Wooldridge are not free from objections. The experimental animals were rabbits, which, as is well known, are

held by many investigators to differ greatly in their susceptibility to anthrax. This difference, however, is denied by Baumgarten. The injection was made apparently by subcutaneous application, which renders the dose employed still more uncertain. Lastly, the control animals, instead of being of the same species, were guinea-pigs, the most susceptible of all animals to anthrax.

After Wooldridge's death, Wright (1891) experimented along the same line, with confirmatory results. He designated the "tissue fibrinogen" as cell fibrinogen, and showed that it yielded on decomposition an albumose body and a nuclein. After injections of this cell fibrinogen, the number of leucocytes in the blood was increased, and the albumose or pepton body appeared in the urine. It may be mentioned here that histon has been reported in febrile urine (Krehl and Matthes, 1895) and in leukæmic urine (Kolisch and Burián, 1896).

The solution of the cell fibrinogen in one-per-cent sodium carbonate was filtered through paper, and afterward through porcelain. This method of sterilization was resorted to in preference to the heating method of Wooldridge. Repeated intravenous injections (2 to 4 cubic centimetres at a time), before and after inoculation with anthrax, gave apparent immunity to four out of fifteen rabbits. Unfortunately, one out of four control rabbits survived. This recovery of a control is hardly due, as Wright was inclined to believe, to a washing away of the germ by a "hæmorrhage at the point of inoculation." As in Wooldridge's experiments, no mention is made of the method of inoculation or the quantity, source, etc., of the culture employed. The crucial test of an established immunity—reinoculation with an otherwise surely fatal dose—was not performed. The life of the treated animals was certainly prolonged (four, five, and six days), but no evidence is brought forward to show that this is not due to the alkalinity of the solution employed.

Brieger, Kitasato and Wassermann, in 1892, attempted to immunize white mice and guinea-pigs from anthrax by a similar process. Their results show a partial immunity in a limited number of instances, but not one of the animals was proof against such



highly virulent material as a bit of the spleen of an animal dead of anthrax.

These authors obtained much more satisfactory results with other pathogenic bacteria. Mixtures of tetanus culture and thymus extract on standing showed diminished toxicity, and mice and rabbits treated with successively increasing doses of such mixtures eventually became perfectly immune from tetanus. The thymus extract not only greatly diminished the production of tetanus toxin, but also favoured its destruction. Cultures of the diphtheria bacillus on thymus bouillon likewise possessed very weak toxic properties, and, after heating fifteen minutes at  $65^{\circ}$  to  $70^{\circ}$  to destroy the toxin, conferred immunity from diphtheria in the majority of the guinea-pigs experimented upon. The cholera vibrio grew well in thymus bouillon, and the cultures possessed considerable toxicity. By heating fifteen minutes at  $65^{\circ}$ , this toxicity was destroyed, and immunity was imparted to guinea-pigs treated with such heated cultures in eighty per cent of the animals experimented on. Similar results were obtained with typhoid cultures in thymus bouillon.

The supposition, apparently justified from these experiments, that the thymus extract possesses specific antitoxic properties is, as pointed out by Behring, erroneous. Modifications in the virulence of a culture must be expected when it grows on such an unfavourable medium as the thymus gland, and, as far as the immunizing results are concerned, subsequent investigations have firmly established the fact that this condition can be obtained by the more or less modified specific toxin. That the toxins can be modified or destroyed by nucleohiston will be seen from the work to follow.

The recent advances in our knowledge of the chemistry of the cell, and more especially of "tissue fibrinogen," made it desirable to investigate the protective power, if any existed, of nucleohiston and of its components histon and nuclein with reference to pathogenic bacteria as well as to bacterial toxins. As already stated, Brieger, Kitasato and Wassermann experimented with what was virtually nucleohiston. The work thus far has covered nucleohiston and histon, and this paper deals with these two compounds.

## NUCLEOHISTON.

*Preparation.*—The method employed was essentially that of Lilienfeld, with such modifications as the means at my disposal necessitated. The perfectly fresh glands, when carefully freed of fat and connective tissue, weighed 10.65 kilos. The glands were cut up by a small sausage machine, and the soft pulp then put through an Enterprise fruit press. The thoroughly expressed connective tissue, etc., amounted to 1,180 grammes. The soft pulp, consisting largely of lymphocytes, was treated with eight to ten volumes of water, and allowed to stand in a very cold room overnight. It was then treated with acetic acid as long as a precipitate continued to form. The precipitate was allowed to settle twenty-four hours, and then the supernatant liquid was removed with a siphon. The precipitate was then washed twice by decantation with a very large quantity of distilled water. The compact pure white precipitate was now treated with an equal volume of a one-per-cent solution of sodium carbonate. It dissolved promptly, but the nucleohiston toward the end of the operation is not so readily soluble. The filtration of the alkaline fluid began in about an hour. On account of the slimy matter present and the large volume of liquid to be filtered (about 100 litres), filtration through paper was not resorted to. Instead an asbestos filter was employed. This was prepared as follows: A Witte's perforated porcelain plate (8 centimetres in diameter) was placed in a funnel, covered with a thin layer of wet muslin; then suction was applied by means of a Chapman aspirator, and suspended asbestos was poured into the funnel. A second layer of muslin was then placed on top, and on this another thin layer of asbestos, and finally a third layer of muslin. As soon as the filter began to clog in the process of filtration, which was assisted by an aspirator, gentle rubbing of the surface layer of muslin with the fingers or a spatula was found to greatly diminish this difficulty. Eventually when the filter did clog, the upper layer of muslin and asbestos was removed, and the lower layers were found to continue the filtration with equally good results.

The clear filtrate thus obtained was treated with acetic acid to



distinct and permanent acid reaction. After settling twenty-four hours, the clear liquid was siphoned off and the precipitate was then washed twice by decantation with large quantities of distilled water. The subsided precipitate was then dissolved in an equal volume of one-per-cent sodium carbonate. This solution could now be filtered through Schleicher and Schüll No. 580 plaited paper without any very great difficulty. A large number of funnels and frequent change of filter paper were all that was necessary. The clear filtrate, on acidification with acetic acid, gave a pure white precipitate, which was allowed to subside and then washed as above. The precipitate was finally transferred to plaited filters (No. 580) and allowed to drain. It was now scraped carefully from the filters and treated with several volumes of absolute alcohol. After standing overnight, the alcohol was decanted and replaced with an equal volume of alcohol and ether. The alcohol-ether liquid was finally decanted, and the precipitate was transferred to a muslin bag and allowed to drain. It was finally spread on previously heated porous porcelain plates and allowed to dry in the air. The dried mass was pulverized and passed through a fine copper sieve. The total yield of the air-dried product from 10.65 kilos of dressed glands was 717 grammes (6.7 per cent).

The following analysis will show the composition of the product prepared by this method. The sample was first extracted in a Soxhlet apparatus with absolute alcohol, then with ether, and finally was dried at 110° to 120° to constant weight.

*Sulphur and Phosphorus Determinations.*—The oxidation of the organic matter was effected after Hammarsten's method. The fused mass was dissolved in water, acidified with 5 cubic centimetres of nitric acid, evaporated to a small volume (about 100 cubic centimetres), and precipitated with barium nitrate. The filtrate and wash water from the barium sulphate was again concentrated, and finally precipitated with ammonium molybdate solution at a temperature of about 60° C. This phosphomolybdate precipitate was then washed as usual with acid ammonium nitrate, dissolved in dilute ammonium hydrate and precipitated with magnesia mixture.



a. 1.0162 grammes of substance gave 0.5073 gramme  $\text{BaSO}_4 = 0.69$  per cent S.; also 0.10263 gramme of  $\text{Mg}_2\text{P}_2\text{O}_7 = 2.82$  per cent P.

b. 1.0328 grammes of substance gave 0.05113 gramme  $\text{BaSO}_4 = 0.68$  per cent S.; also 0.10483 gramme of  $\text{Mg}_2\text{P}_2\text{O}_7 = 2.83$  per cent P.

*Nitrogen Determination.*—This was determined in the usual manner by Kjeldahl's method.

a. 0.3181 gramme of substance neutralized 38.1 cubic centimetres of  $\frac{\text{N}}{10}$  oxalic acid (corrected for blank). The calculated amount of nitrogen is 0.5334 gramme = 16.77 per cent N.

b. 0.3076 gramme of substance neutralized 36.55 cubic centimetres of  $\frac{\text{N}}{10}$  oxalic acid (corrected for blank). The calculated amount of nitrogen is 0.5117 gramme = 16.63 per cent N.

	Lilienfeld.	Brieger, Kitasato and Wassermann.	Novy.
S. per cent. ....	0.70	...	0.69
P. " ....	3.02	2.9	2.83
N. " ....	16.86	...	16.70

When ignited in a platinum dish, it yielded 2.78 per cent ash, which contained chiefly phosphoric acid and iron. The presence of iron in nucleohiston was pointed out by Wooldridge and by Lilienfeld. Brieger, Kitasato and Wassermann obtained 1.07 per cent ash from their "active principle" of the thymus gland.

The *chemical properties of nucleohiston* may be briefly summarized: When freshly precipitated and still moist, it is readily soluble in excess of acetic acid (also in 0.4 per cent  $\text{HCl}$ , Wright), in dilute neutral salt solution, in dilute sodium carbonate, sodium phosphate, sodium hydrate, in dilute acids, ammonium hydrate and ammonium sulphide. According to Lilienfeld, as well as Krehl and Matthes, on treatment with alcohol it becomes insoluble in sodium carbonate, in salts and in acids. This is true of the air-dried nucleohiston, whether treated with alcohol or not. In this condition it can be readily brought into solution by the addition of a few drops of sodium hydrate.

Nucleohiston is insoluble in water, alcohol, ether and benzol; the neutral solution or even the sodium-carbonate solution is precipitated by carbonic acid; also by acetic and hydrochloric acids, platinum chloride, silver nitrate and mercuric chloride. The solution is not precipitated with magnesium sulphate or sodium chloride, nor does it gelatinize with sodium hydrate or salts. The solution in sodium hydrate or carbonate treated with alcohol gives a precipitate of the sodium compound which is readily soluble in water, and treated with barium chloride gives the barium compound.

Nucleohiston gives the Millon and xanthoproteic reactions, also a faint biuret reaction on standing. It decomposes peroxide of hydrogen, but this property is lost on treatment with alcohol and ether. Digested with pepsin-hydrochloric acid, it yields an abundant precipitate of nuclein, containing 4.99 per cent of phosphorus. On boiling with water, it yields a nuclein, which is soluble, and histon. Treated with calcium hydrate, a precipitate of Ca-histon forms, whereas the nuclein remains in solution. Barium hydrate, on the other hand, gives a precipitate of barium nuclein, and the histon remains in solution. The nucleohiston is also readily split up by the action of acids, and, if heated, yields nucleinic acid and, finally, adenin, guanin, cytosin, etc.

The action of nucleohiston on the blood in rabbits and dogs has been referred to. I have been able to obtain similar results in rabbits. Krehl and Matthes found that a subcutaneous injection of 0.5 gramme of nucleohiston (unlike albumose) in guinea-pigs produced no fever. The same dose in tubercular guinea-pigs was also without effect on the temperature, but death followed in a short time with or without a local reaction.

Contrary to the above statements, I have found that intravenous or subcutaneous injections of 0.3 gramme of nucleohiston produce in rabbits (to a less extent in guinea-pigs), after a temporary subnormal temperature, a rise of  $1^{\circ}$  to  $1.5^{\circ}$  C. The febrile condition may last several days. After several injections, the animals scarcely react.

According to Freund and Grosz, nucleohiston precipitates the diphtheria toxin quantitatively, and in this respect resembles nucleinic



acid. It also is said to precipitate antitoxin from serum. This precipitation of toxin or of antitoxin is clearly not direct, but rather a mechanical dragging down of these active constituents in the same way as this is done by a precipitate of calcium phosphate. The addition of hydrochloric acid as practised in both cases results in the precipitation of nucleohiston, and with this are carried down the substances mentioned. This criticism also applies to Tichomiroff's precipitation of toxins with nucleinic acid, which readily forms a precipitate with proteids.

#### NUCLEOHISTON AND TETANUS TOXIN.

The powdered nucleohiston, as already stated, is with difficulty soluble in water. A strong aqueous solution can not, therefore, be prepared directly. The procedure followed in this and other instances was to add a few drops of strong potassium-hydrate solution to the suspension of nucleohiston in water. The compound dissolves quite easily, especially on standing. The solution of nucleohiston is then filtered and precipitated by slightly acidulating with acetic acid. The precipitate is allowed to settle and the liquid decanted. The precipitate is then washed once or twice by decantation, and finally transferred to a filter. As soon as the liquid has drained off, the moist precipitate is stirred into a one-per-cent solution of sodium carbonate containing a few drops of alkali. Freshly precipitated nucleohiston dissolves quite readily in sodium carbonate. In this way a ten-per-cent milky opalescent solution can be obtained. For use, this was usually diluted with an equal volume of distilled water, thus giving a five-per-cent nucleohiston solution in 0.5-per-cent sodium carbonate. Any free alkali present is fixed by passing carbonic acid through the solution until it begins to yield a precipitate.

The tetanus toxin was prepared by filtering the culture of the bacillus in glucose bouillon through porcelain. A minute amount of tricresol was added to the toxin.

Only guinea-pigs were employed in the work with tetanus toxin. The injections were made subcutaneously and separately, with but a slight interval of time between. Guinea-pigs 56 and 57 received one

half of the nucleohiston intraperitoneally, the other half subcutaneously.

TABLE I.

Guinea-pig No.	Weight.	Nucleohiston.	Tetanus toxin.	Death, in hours.
55	290	250 mg.	0.1 c. c.	50
56	310	500 "	0.1 "	40
57	375	500 "	0.1 "	50
Control 58	416	...	0.1 "	46

The nucleohiston, therefore, under these conditions does not protect against tetanus toxin.

In the following experiment, 0.5 cubic centimetre of tetanus toxin was mixed with 5 cubic centimetres of a five-per-cent nucleohiston solution; 1.1 cubic centimetres of the mixture, therefore, contained 0.1 cubic centimetre of tetanus toxin. This quantity of the mixture was injected subcutaneously in guinea-pigs at intervals indicated in the table. The mixture was kept in an ice chest.

TABLE II.

Guinea-pig No.	Weight.	Dose.	Mixture used.	Death, in hours.
Control 58	416	0.1 c. c. toxin.	.....	46
59	325	1.1 " mixture.	At once.	50
60	200	1.1 " "	After 24 hours.	44
61	265	1.1 " "	" 48 "	48
62	250	1.1 " "	" 96 "	No effect.

From this it is seen that the nucleohiston solution does not weaken or destroy the tetanus toxin until after contact for two days. The mixture four days old failed to kill the guinea-pig, but conferred no immunity, since when inoculated eight days later with 0.1 cubic centimetre of tetanus toxin the animal died in thirty-two hours. On comparing this table with Table IV, it will be seen that the diphtheria toxin is destroyed by the nucleohiston solution much more rapidly than is the tetanus toxin. A similar relative weakness of the diphtheria toxin to formalin has been pointed out by Burckhard. The quantity of formalin sufficient to destroy the diphtheria toxin was not sufficient for the tetanus toxin. In other words, the tetanus toxin, which is often considered as the most unstable toxin, is with



reference to certain chemicals more permanent than the diphtheria toxin. The action of the nucleohiston solution is not due wholly, or even in great part, to the alkali present, as can be seen in the experiments with diphtheria toxin.

#### NUCLEOHISTON AND DIPHTHERIA TOXIN.

In the following experiment the injections were made separately. Guinea-pigs 64 and 65 received two injections, each of 250 milligrammes of nucleohiston, one of which was given subcutaneously, the other intraperitoneally.

TABLE III.

Guinea-pig No.	Weight.	Nucleohiston.	Diphtheria toxin.	Death, in hours.
63	260	250 mg.	0.1 c. c.	80
64	...	500 "	0.1 "	40
65	...	500 "	0.1 "	40
Control 66	230	.....	0.1 "	60

As in Experiment I with tetanus toxin, nucleohiston is without any immunizing or protecting action toward the diphtheria toxin.

In the following table are tabulated the results obtained with a mixture of 5 cubic centimetres of nucleohiston and 0.5 cubic centimetre of diphtheria toxin; 1.1 cubic centimetres of this mixture contained 0.1 cubic centimetre of diphtheria toxin. The mixture was kept in the ice chest. The injections were made under the skin.

TABLE IV.

Guinea-pig No.	Weight.	Dose.	Mixture used.	Death, in hours.
Control 53	...	0.1 c. c. toxin.	.....	49
67	285	1.1 " mixture.	At once.	64
68	225	1.1 " "	After 24 hours.	No effect.
69	245	1.1 " "	" 48 "	"
70	250	1.1 " "	" 96 "	"

From this it is seen that the nucleohiston solution destroys the diphtheria toxin in less than twenty-four hours. In the case of tetanus, as seen in Table II, the toxin is destroyed after the forty-eighth and before the ninety-sixth hour. This shows, as already pointed out, that the tetanus toxin is more resistant than the diphtheria

toxin to the action of nucleohiston. The animals (68, 69, 70) that survived the injection were not rendered immune, since an injection with 0.1 cubic centimetre of diphtheria toxin, made eleven, ten, and eight days respectively after the first injection, caused death in forty-eight, thirty-two, and forty-eight hours respectively.

This destruction of the tetanus and diphtheria toxin by the nucleohiston solution may be due in part to the sodium carbonate. The alkali, however, in double the strength employed weakens the toxin much more slowly than does the nucleohiston solution. This action of sodium carbonate can be seen from the following experiments. For this trial 0.8 cubic centimetre of diphtheria toxin was added to 4 cubic centimetres of a one-per-cent solution of sodium carbonate; 1.2 cubic centimetres of the mixture therefore contained 0.2 cubic centimetre of toxin. The first animal was inoculated after the mixture had been allowed to stand for one hour. The latter was then placed in the ice chest and inoculations were made as indicated.

TABLE V.

Guinea-pig No.	Weight.	Dose.	Mixture used.	Death, in days.
Control 71	420	0.1 c. c. toxin.	.....	2½
“ 72	514	0.2 “ “	.....	3½
78	422	1.2 “ mixture.	1 hour old.	1½
79	395	1.2 “ “	52 hours old.	7½
90	244	1.2 “ “	100 “ “	No effect.

This shows that while a nucleohiston solution containing one half per cent sodium carbonate destroys the toxin within twenty-four hours, a one-per-cent sodium-carbonate solution does not destroy the toxin, but weakens it in about two days. The toxin used was the same as that employed before, but was weakened by age; hence a larger dose was used.

#### NUCLEOHISTON AND HOG CHOLERA.

The bouillon cultures of the hog-cholera bacillus employed for inoculation were always from two to three days old. The injection of nucleohiston was in some cases repeated two or three times at intervals of two days.



TABLE VI.

Rabbit No.	Weight.	Nucleohiston.	Hog-cholera bouillon.	Death, in days.
60	1,230	250 mg. intravenously.	0.25 c. c.	3½
61	1,580	250 " subcutaneously.	0.25 "	6½
62	2,000	400 " intravenously in 2 injections.	0.25 "	3
63	1,580	750 " subcutaneously in 2 injections.	0.25 "	5½
64	2,100	1.0 g. intravenously in 3 injections.	0.25 "	4
65	1,800	1.25 g. subcutaneously in 3 injections.	0.25 "	5
Control 66	1,550	.....	0.25 "	2½

It would appear from this set that nucleohiston at least apparently retards death from the hog-cholera bacillus. Recognising, however, the variation that exists in the susceptibility of rabbits to this germ, the conclusion is justified that nucleohiston does not protect against hog cholera; 2.5 grammes of nucleohiston introduced into a rabbit (67) in four injections, three, twenty-four, thirty-six, and seventy hours after inoculation with 0.25 cubic centimetre of hog-cholera culture, did not prevent death, which ensued in three days and a half.

#### NUCLEOHISTON AND ANTHRAX.

It has been pointed out in the introduction that with "tissue fibrinogen" or "cell fibrinogen" Wooldridge and Wright believed that they obtained immunity from anthrax. Wooldridge had apparent success in two and Wright in four cases. There is no evidence, as pointed out, that immunity really was established, since reinoculation was not resorted to. In the following experiments only one rabbit (No. 53) out of twelve animals was protected against the injection of an anthrax culture. On subsequent inoculation, however, eleven days later, the rabbit died of anthrax in three days and three quarters. This rabbit received intravenously 400 milligrammes of nucleohiston. During the next twenty-four hours the temperature rose to 40.5°. On the second day it returned to the normal, 38.9° to 39.5°, and on the third it was subnormal, 37.3° to 38°. At this point the rabbit was inoculated with 0.1 cubic centimetre of a fresh bouillon culture of anthrax. Rabbits Nos. 51 and 52 were treated in exactly the same way. Their temperature before inoculation, however was 39.4° and 39.6°.

The injections were made separately, except in guinea-pigs 50

and 51, where a mixture of 0.3 cubic centimetre of anthrax culture and 2.7 cubic centimetres of a five-per-cent nucleohiston was employed. One cubic centimetre of this mixture therefore contained 0.1 cubic centimetre of the anthrax culture. The anthrax injections were in all cases made subcutaneously, and, unless otherwise indicated, were made immediately after the injection of the nucleohiston.

With the exception of Rabbit 53, the results, as seen from Table VII, are sufficient to show that nucleohiston possesses no specific action with reference to anthrax.

TABLE VII.

Animal No.	Weight.	Nucleohiston.	Anthrax.	Death, in days.
Rabbits.				
Control 55	1,570	.....	0.1 c. c.	3 $\frac{1}{2}$
51	1,440	0.3 gm. intravenously.	0.1 " 2 days later.	3 $\frac{1}{4}$
52	1,389	0.5 " "	0.1 " 2 " "	3 $\frac{1}{2}$
53	1,590	0.4 " "	0.1 " 2 " "	Recovered.
56	1,200	0.675 gm. intravenously, 3 injections in 8 days.	0.1 " 3 " "	2 $\frac{1}{2}$
57	1,090	1 gm. subcutaneously, 3 injections in 8 days.	0.1 " 3 " "	3
58	1,050	0.5 gm. subcutaneously.	0.1 "	2
59	1,670	0.3 " intravenously.	0.1 "	2
Guinea-pigs.				
Control 47	824	.....	0.1 "	1 $\frac{1}{2}$
46	490	0.3 gm. subcutaneously.	0.1 " 2 days later.	4 $\frac{1}{4}$
48	600	0.5 " "	0.1 "	1 $\frac{3}{4}$
49	660	1 gm. subcutaneously, 3 injections in 8 days.	0.1 " 3 days later.	1 $\frac{1}{2}$
50	758	1 c. c. of anthrax-nucleohiston mixture, 4 days on ice.	.....	2 $\frac{1}{2}$
51	800	1 c. c. of anthrax-nucleohiston mixture, 11 days on ice.	.....	3

## HISTON.

The term histon was employed first by Kossel to designate an albumose or peptonlike body which he found in the red blood cells of geese. He showed that it did not exist as such in the cells, but in combination with another body, presumably nuclein. In 1891 Wright showed that "tissue fibrinogen" readily split up into an albumose half and a nuclein half. The injection of "tissue fibrinogen" in rabbits and dogs increased the number of leucocytes, and in the urine he found albumose or pepton. As already pointed out, histon has been found during the past year in febrile and leukæmic



urines. The researches of Lilienfeld have shown that "tissue fibrinogen" is largely made up of nucleohiston, which readily yields nuclein and histon.

Histon may be prepared from nucleohiston or from the gland direct. The nucleohiston (50 grammes) is rubbed up in a mortar with 250 cubic centimetres of 0.8-per-cent HCl (8 cubic centimetres of 1.20 specific gravity HCl to 1,000 cubic centimetres of water). After standing twenty-four hours, the liquid is decanted and filtered, and the filtrate precipitated with six to eight volumes of a mixture of equal parts of absolute alcohol and ether. The precipitate is filtered, washed with ether, then pressed between filter paper, and finally allowed to dry in the air. The dried residue, powdered, is pure white and chalky in appearance. The residue after the first extraction can be treated with the dilute hydrochloric acid several times in succession, and the filtrate from each treatment yields an additional quantity of histon. The product, however, was at first yellowish, waxy in character, and when dried gave a yellowish powder. The total yield of histon in this case was nearly 5 grammes.

The filtration in this method is very slow, and it is much more convenient to resort to centrifugation, in which case the yield of histon is better. The yield is usually about ten per cent of the air-dried, powdered nucleohiston.

Histon was also prepared direct from the fresh thymus glands. The glands, freed from connective tissue, etc., were passed through an Enterprise fruit press. The thick pulp thus obtained was treated with two volumes of water, and set aside at 39° for two hours, with frequent stirring. The slimy character is thus greatly diminished, and the material brought quickly into solution. Hydrochloric acid (one per cent) was then added, and the liquid stirred frequently during the next four hours. The mixture was then centrifugated, and the clear, opalescent liquid was decanted and filtered through paper. This liquid, precipitated with alcohol and ether, as in the preceding method, gave a yellowish, semi-crystalline powder. The yield was about 3.6 per cent of the fresh gland.

The *reactions* of histon can be briefly summarized. It is readily

soluble in water, and the slightly opalescent solution thus obtained is distinctly acid. The solution is precipitated on saturation with sodium chloride, sodium carbonate, ammonium chloride, ammonium sulphate, and magnesium sulphate. This latter fact is of importance, since it shows a relationship to the globulins which, as will be seen presently, possess a similar action on toxins. A white, flocculent precipitate is also given by ammonium hydrate. If the precipitate is allowed to subside and stand for a while, it becomes insoluble in excess of reagent, whereas if an excess of ammonia is added at once the precipitate that forms redissolves immediately. According to Kossel, this ammonia precipitate is insoluble, but it is soluble in HCl (Lilienfeld) and in acetic acid (Kolisch and Burián). Calcium- and sodium hydrate also yield precipitates. Barium hydrate does not give a precipitate. Nitric acid gives a precipitate which disappears on heating and reappears on cooling (albumose reaction). The concentrated solution is also precipitated by alcohol.

Histon is not precipitated by calcium or mercuric chlorides, neutral or basic lead acetate, sodium phosphate, acetic or sulphuric acids. On boiling, according to Kossel, there is no coagulation, whereas Lilienfeld and Kolisch and Burián state that it is coagulated. The coagulum is soluble in mineral acids. On prolonged heating with baryta, it yields leucin and tyrosin. It does not contain phosphorus.

It gives the biuret reaction in the cold. This fact, together with its behaviour to nitric acid and its peculiar action on blood, led Lilienfeld to consider this body as an albumose. Its behaviour to magnesium sulphate and to heat shows a relation to globulins.

As is well known, Schmidt-Mülheim, and later Fano, showed that intravenous injections of 0.3 gramme of pepton, per kilo body weight, prevent coagulation of blood. Pollitzer eventually demonstrated that this property does not belong to true pepton, but rather to hetero-albumose. Intravenous injections of histon, as pointed out by Lilienfeld, give a similar non-coagulable condition of the blood. Histon-plasma, however, is different in many respects from pepton-plasma. With the histon prepared as above, I have been able to obtain characteristic histon-plasma as described by Lilienfeld. Histon, however,



is quite poisonous, and this toxic action is not due to the acid reaction of the solution, as Lilienfeld is inclined to believe. It is possible to inject intravenously, with only temporary effects, into rabbits many times the amount of free HCl corresponding to the acidity of the histon solution.

The several preparations of histon possessed, as stated, decided toxic properties. In guinea-pigs weighing 300 grammes or under, 100 milligrammes frequently produced marked intoxication, whereas 200 milligrammes often produced death. Repeated injections of small doses failed to establish a tolerance. Indeed, an increased susceptibility was observed in animals after two or three injections, so that a dose of 100 milligrammes eventually sufficed to produce death in a few hours. In animals that recovered from larger injections, a necrotic area would form at the point of inoculation.

Subcutaneous injection in guinea-pigs of 200 milligrammes produced a slight temporary depression of temperature, giving place in a few hours to an increase of  $1^{\circ}$  to  $1.5^{\circ}$  C., which would persist for forty-eight hours or longer.

Exceedingly rapid effects were brought out in rabbits with intravenous injections of histon. In this case a dose of 100 milligrammes of histon, whether acid or neutral, was dangerous, and often produced death in a few minutes. In such cases the heart was very irregular, respiration laboured, and the animal would lie in a helpless condition, with eyes moist, and often with symptoms of salivation.

The histon prepared according to the methods given is probably far from being a chemically pure product. The white chalky powder gave 0.7 per cent ash, whereas the histon prepared directly from the gland by the second process gave 1.45 per cent ash, which contained some chlorine and much phosphoric acid. This is further seen in the differences in reactions between the preparations made by the several workers and in the wholly unlike results obtained by experimentation on animals. The anti-coagulating power appears to be constant; but, on the other hand, apparently irreconcilable results have been obtained as to toxicity, as to thermogenic effects, and, above all and of most importance, as to immunizing properties. The immunizing

power of nucleohiston, as described by Wooldridge and by Wright, has been shown from the preceding work not to exist. The same may be said, in advance, to be true of histon.

The most noteworthy contributions in this direction have been made by Freund, Grosz and Jelinek. These authors have endeavoured to show a relation between blood coagulation and the action of antitoxins; in other words, that histon, which possesses anticoagulating powers, is likewise antitoxic in action. A single subcutaneous injection of 100 milligrammes or more of histon was sufficient to protect against a fatal dose of diphtheria toxin (0.2 cubic centimetre). Likewise an injection of 400 milligrammes of histon, followed twenty-four hours later by an injection of 0.15 cubic centimetre of a two-day diphtheria culture, was without effect, although the control died in two days. My experiments with histon have failed to confirm not only this protective action against the toxin, but also the harmlessness of injections of 400 to 500 milligrammes of histon. Half that amount, indeed, is sufficient, when introduced subcutaneously, in guinea-pigs, to produce death in a few hours.

Freund and Grosz, in a recent paper, record similar results with albumoses as with histon. Thus, an injection of 100 to 210 milligrammes of deutoalbumose, or of 50 to 100 milligrammes of protalbumose, sufficed to protect against a fatal dose of diphtheria toxin. Ten milligrammes of either deutoalbumose or protalbumose protected mice against a fatal dose of tetanus toxin (0.005 cubic centimetre), whereas 100 to 150 milligrammes of protalbumose protected rabbits against the same poison. It is needless to say that results like this, obtained with such small quantities of albumose, are open to question. Witte's pepton, which is largely made up of albumose, can be expected to give similar results, but thus far they have not been satisfactory.

In this connection attention may be called to the somewhat similar work of Smirnow, who found that 0.02 cubic centimetre and upward of a 1.1-per-cent solution of globulin from a normal serum of a horse, mixed with a fatal dose of diphtheria toxin, neutralizes the poison. Such a mixture when injected is without effect, but if the



globulin and the toxin be injected separately in different parts of the body, death follows. These results are not above suspicion, for it is altogether likely that something more than 0.0002 gramme of globulin is necessary to protect against the diphtheria toxin.

In the work with histon, as a rule, a ten-per-cent solution was employed. The histon solution is distinctly acid, 100 milligrammes requiring about 0.5 cubic centimetre of a one-per-cent solution of sodium carbonate for neutralization. In some instances the histon solution was neutralized before injection. As in the preceding experiments, the injections of histon and of toxin were made separately, subcutaneously, and in different places, unless otherwise indicated. About four different preparations of histon were employed, without any difference in the results.

### HISTON AND TETANUS TOXIN.

TABLE VIII.

Guinea-pig No.	Weight.	Histon.	Tetanus toxin.	Death, in days.
Control 2	280	.....	0.1 c. c.	3
3	...	0.1 gm.	0.1 "	6 $\frac{1}{2}$
4	...	0.2 "	0.1 "	6 $\frac{1}{2}$
19	252	0.2 " *	0.1 "	3 $\frac{1}{2}$
20	330	0.1 " †	0.1 "	9
41	...	0.1 " neutral.	0.25 "	1 $\frac{1}{2}$
42	...	0.1 " "	0.25 "	1 $\frac{1}{8}$
Control 43	...	.....	0.25 "	1 $\frac{1}{2}$

\* On each of the two following days after the injection of the tetanus toxin an additional injection of 0.2 gramme of histon was made.

† Two injections of 0.1 gramme each, as in the preceding.

The first five animals received an old tetanus toxin solution, of which 0.1 cubic centimetre closely approximated the minimum fatal dose. The last three received another and more recent toxin. The failure of histon to protect animals from an intoxication with the tetanus poison is thus shown.

A fifteen minutes' old mixture of the toxin and histon, whether acid or neutral, does not save guinea-pigs. Death occurs about the same time as with controls. In a similar mixture of diphtheria toxin and acid histon the toxin is destroyed within five minutes. This again shows that the diphtheria toxin is less resistant than the tetanus toxin.

## HISTON AND DIPHTHERIA TOXIN.

The following experiments with diphtheria toxin furnished the same result as with tetanus toxin. Separate injections of histon and of diphtheria toxin do not protect against the latter.

TABLE IX.

Guinea-pig No.	Weight.	Histon.	Diphtheria toxin.	Death, in hours.
Control 6	200	.....	0·3 c. c.	24
7	115	0·1 gm.	0·3 "	29
8	297	0·2 "	0·3 "	27
Control 11	252	.....	0·1 "	30
9	290	0·2 gm. in 2 injections.	0·1 "	41
13	274	0·3 " " 3 "	0·1 "	36
35	287	0·1 "	0·06 "	60
36	160	0·1 "	0·06 "	36
Control 37	357	.....	0·06 "	72
" 41	232	.....	0·06 "	36
44	185	0·1 gm. neutral.	0·06 "	36
45	170	0·1 "	0·06 "	36
Rabbit 27	Control	.....	0·5 "	42
" 29	1,560	0·08 gm. intravenously.	0·5 "	48
" 36	1,920	0·18 " "	0·7 "	52

These results on rabbits and guinea-pigs are wholly unlike those of Freund, Grosz and Jelinek. In not a single instance was the animal's life prolonged, much less saved. Larger doses of histon than those given could not safely be employed, owing to the toxic action of the substance.

In the preceding experiments the injections of histon and of toxin were made separately in different parts of the body. As pointed out, the results are wholly negative, and consequently diametrically opposite to those of Freund, Grosz and Jelinek. A mixture of histon and toxin, however, when injected into guinea-pigs is not fatal, as the following trials indicate: The mixture was prepared by adding 0.8 cubic centimetre of the diphtheria toxin to 4 cubic centimetres of a ten-per-cent histon solution; 1.2 cubic centimetres of the mixture, therefore, contained 100 milligrammes of histon and 0.2 cubic centimetre of toxin, which represents a surely fatal dose. The toxin was the same as used in preceding experiments, but, owing to the lapse of several weeks, it had become less active. The histon solution was



decidedly acid, 1 cubic centimetre of the ten-per-cent solution requiring 1.2 cubic centimetres of  $\frac{N}{10}$  KOH for neutralization. The injections were subcutaneous.

TABLE X.

Guinea-pig No.	Weight.	Dose.	Age of mixture.	Death, in days.
77	420	1.2 c. c.	1 hour.	No effect.
80	505	1.2 "	52 hours.	" "
85	505	1.2 "	5 minutes.	" "
87	510	1.2 "	1 hour 40 min.	" "
Control 71	420	0.1 " toxin.	.....	2 $\frac{1}{4}$
" 72	514	0.2 " "	.....	3 $\frac{1}{8}$
" 89	535	0.2 " "	.....	2 $\frac{1}{2}$

A mixture of the toxin and histon becomes non-poisonous within a few minutes. Is this failure of the toxin to act due to an immunizing action of the histon, or is it due to a direct action of the histon on the toxin, neutralizing or destroying the latter? Or, thirdly, is the toxin destroyed by the acidity of the histon solution? As for the first supposition, it is evident from the preceding experiments that histon solutions, either neutral or acid, fail to immunize. Furthermore, the animals that recovered from an injection of the mixture were not immune to a subsequent injection of toxin.

As for a direct action of the histon on the toxin, this is possible, especially if it be remembered that histon resembles albumoses and globulins in certain reactions. Ordinary albumoses and serum globulin of the horse have been reported to protect in mixture against the diphtheria toxin.

The strongly acid character of the histon solution must undoubtedly exercise a destructive action on the unstable diphtheria toxin. A mixture of 1.2 cubic centimetres of  $\frac{N}{10}$  HCl and 0.2 cubic centimetre of the toxin, after standing one hour and forty minutes, was injected into a guinea-pig with no ill effects following. Indeed a similar mixture five minutes old is without effect. Furthermore, a neutral histon solution mixed with the toxin and injected five minutes later into a guinea-pig produced death in forty-four hours—the same time as for a control. It would appear from this that the destructive action of

histon on the diphtheria toxin is largely, if not wholly, due to the hydrochloric acid present.

#### HISTON AND HOG CHOLERA.

In the following experiments fresh cultures in bouillon of the hog-cholera bacillus were employed. The injections were all subcutaneous:

TABLE XI.

Rabbit No.	Weight.	Histon.	Hog cholera.	Death, in days.
1	635	0.1 gm. neutral.	0.1 c. c.	4 $\frac{3}{8}$
2	940	0.2 " "	0.1 "	4 $\frac{1}{8}$
Control 3	1,295	.....	0.1 "	2 $\frac{1}{2}$
4	1,020	0.8 gm. neutral, 5 inj. in 5 days.	0.1 "	5 $\frac{1}{2}$
5	895	0.14 gm.	0.1 "	3

The results from histon injections in hog cholera are the same as those with nucleohiston.

In this connection we may add that unsuccessful attempts were made to produce immunity in rabbits with thymus bouillon cultures of the hog-cholera germ heated to 56°. The thymus bouillon culture therefore offers no advantage over the ordinary bouillon cultures.

#### HISTON AND ANTHRAX.

The anthrax culture used for the following experiments was prepared and employed in the same manner as in the experiments with nucleohiston:

TABLE XII.

Rabbit No.	Weight.	Histon.	Anthrax.	Death, in days.
41	1,575	0.1 gm. intravenously.	0.1 c. c.	4 $\frac{1}{2}$
42	2,120	0.2 " "	0.1 "	3 $\frac{1}{2}$
Control 43	835	.....	0.1 "	1 $\frac{5}{6}$
44	1,110	0.1 gm. neutral intrav.	0.1 "	1 $\frac{1}{2}$
45	1,550	0.1 " " "	0.1 "	2 $\frac{1}{2}$
46	1,590	0.1 " " "	0.1 "	2 $\frac{1}{2}$
48	1,800	0.1 " " subcut.	0.1 "	3 $\frac{1}{2}$
49	1,670	0.1 " " "	0.1 "	5 $\frac{1}{2}$
Control 50	1,800	.....	0.1 "	3
Guinea-pig 50	....	0.1 gm.	0.1 "	2
Control 51	....	.....	0.1 "	1 $\frac{1}{2}$
52	....	0.15 gm. in 2 injections.	0.1 "	1 $\frac{1}{2}$
53	320	0.15 " " 2 "	0.1 "	1 $\frac{1}{2}$



The above table clearly shows that histon, like nucleohiston, is without any protective action against the anthrax bacillus.

#### CONCLUSIONS.

The results obtained in the preceding experiments may be briefly summarized as follows:

1. Nucleohiston does not protect against a separate and subcutaneous injection of tetanus toxin, diphtheria toxin, hog-cholera bacillus, or anthrax bacillus.

2. Mixtures of nucleohiston and either tetanus toxin or diphtheria toxin lose their specific toxic action—the latter much more rapidly than the former. Animals that recover after inoculation of such mixtures are not rendered immune.

3. In a mixture of nucleohiston and anthrax bacillus the latter is unaffected, even after the lapse of eleven days.

4. The destruction of specific toxins in a solution of nucleohiston, though in part due to sodium carbonate, is probably chiefly due to the nucleohiston.

5. Histon does not protect against a separate and subcutaneous injection of tetanus toxin, diphtheria toxin, hog-cholera bacillus, or anthrax bacillus.

6. In a mixture of histon and diphtheria toxin the latter is destroyed in a few minutes. This action is in part, if not wholly, due to the acidity of the histon solution. Similar mixtures of closely related bodies, serum globulin or albumoses, in Witte's pepton, give analogous results. The animals that recover from such inoculations are not rendered immune. Histon does not destroy the tetanus toxin as readily as the diphtheria toxin.

7. Histon possesses decided and marked toxic properties which are not due to the hydrochloric acid present.

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